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Morphological and physiological responses to drought stress of carob trees in Mediterranean ecosystems

Khouloud ZAGOUB, Khouloud KRICHEN, Mohamed CHAIEB, Lobna F MNIF[*]

Laboratory of Ecosystems and Biodiversity in Arid Land of Tunisia, Faculty of Sciences, University of Sfax, Sfax 3000, Tunisia

Abstract: The greatest failure rate of reforestation programs is basically related to water deficit, especially at the seedling stage. Therefore, the main objective of this work is to investigate the responses of three accessions of carob trees (*Ceratonia siliqua* L.) with 2-year-old from different climate regions to drought generated by four water treatments: Tc (250 mm), T1 (180 mm), T2 (100 mm), and T3 (50 mm). The first accession (A1) comes from the protected national park of Ichkeul in northern Tunisia. This zone belongs to the bioclimatic sub-humid stage. The second accession (A2) comes from Melloulech, located in the center-east of Tunisia, belonging to the bioclimatic semi-arid stage. The third accession (A3) comes from the mountain of Matmata, located in the south of Tunisia, belonging to the bioclimatic hyper-arid stage. The experiment was undertaken in a greenhouse. Gaz exchange indices (net photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration (C_i)) were determined. Predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potentials, relative soil water content (SWC), and morphological parameters (plant height (H), number of leaves (NL), number of leaflets (Nl), and number of branches (NB)) were estimated. The results showed that significant differences ($P<0.001$) were found between physiological and morphological parameters of each accession. The highest growth potential was recorded for Tc treatment in both accessions A1 and A2. Significant decreases in g_s, E, C_i, and SWC were recorded with the increases in water stress applied from treatment T1 to T3. Positive and significant correlations were found between SWC and Ψ_{pd} for all studied accessions. Ψ_{pd} and Ψ_{md} decreased as water stress increased, ranging from –0.96 to –1.50 MPa at sunrise and from –1.94 to –2.83 MPa at midday, respectively, under control and T3 treatments. *C. siliqua* accessions responded to drought through exhibiting significant changes in their physiological and morphological behavior. Both accessions A1 and A2 showed greater drought tolerance than accession A3. These seedlings exhibit different adaptive mechanisms such as stress avoidance, which are aimed at reducing transpiration, limiting leaf growth, and increasing root growth to exploit more soil water. Therefore, *C. siliqua* can be recommended for the ecological restoration in Mediterranean ecosystems.

Keywords: *Ceratonia siliqua* L.; water stress; soil moisture; stomatal conductance; predawn leaf water potential; minimum water potential

1 Introduction

Due to human activities, such as overgrazing, fires, logging, and uncontrolled urbanization, almost all countries of the Mediterranean have undergone large-scale destruction of their habitats

(Ozturk et al., 2008). In addition to the harmful effects of anthropogenic activities, abiotic stress plays an intrinsic role in terms of decreased productivity and plant development (Qiao et al., 2011). Indeed, heat, cold, salinity, and drought cause more than 50% of crop losses each year (Qiao et al., 2011). This decrease in yield depends not only on the duration and intensity of stress, but also on the development stage of the plant (Shao et al., 2008). Therefore, the consequences of this stress favour the emergence of biotic stress. Water deficits and increased temperatures, which affect plant productivity, can occur simultaneously. Intergovernmental Panel on Climate Change (IPCC, 2001) asserted that global climate change scenarios indicate further warming from 1.8°C to 4.0°C by 2100, with variations in precipitation pattern, as atmospheric concentrations of greenhouse gases and carbon dioxide (CO_2) increase. As a result of these scenarios and by exposing plants to warmer climatic conditions, the photosynthetic response to water stress may be altered (Chaves et al., 2003).

In arid and semi-arid Mediterranean ecosystems, one of the most severe abiotic stressors is drought, which negatively affects plant development and growth (Qiao et al., 2011; Zagoub et al., 2022). Shao et al. (2008) argued that soil drought leads to water deficits in leaf tissues, thus influencing multiple physiological processes. Drought significantly reduces germination, referring mainly to decreased water uptake during imbibition phase, low energy supply, and decreased enzyme activity (Okçu et al., 2005). It also entails a reduction in leaf size, resulting in a decrease in photosynthetic surface area and stem elongation, in addition to a decrease in root propagation (Li et al., 2009). Furthermore, it plays a crucial role in modifying stomatal variations, as well as the water and nutrient relations of plant, which consequently produces a decrease in crop productivity (Li et al., 2009). Chaves et al. (2003) demonstrated that a water deficit impacts stomatal opening and cell turgor, resulting in reduced photosynthetic and transpiration rates. In fact, CO_2 assimilation is strongly reduced, limiting leaf metabolism. This situation gives rise to a regression of forest cover in an irreversible way, with dangerous environmental consequences, such as rapid soil erosion and desertification (Mokhtar et al., 2022). The drought becomes more severe, more prolonged, and more frequent in these regions, which alters the vegetation biodiversity (Gullo et al., 2003).

Following the increase in desertification related to the decline of vegetation and the expansion of arid and semi-arid areas, Collins et al. (2013) addressed the regression of Mediterranean environment. These problems affect ecosystem functioning and result in forest cover degradation (Valencia et al., 2015). According to 6th IPCC (2021), Tunisia is one of the most vulnerable countries, namely to climate change impacts, some of which being irreversible. The climate change effect is mainly due to the geographical location of Tunisia, as it lies between intertropical and temperate regions of northern hemisphere, which makes its climate particularly variable. For these reasons, to restore forest ecosystems, planting multipurpose agroforestry species that are not only tolerant to drought but also possess morphophysiological characteristics and genetic adaptation to climatic variations is recommended. Among these species, *Ceratonia siliqua* L. is an evergreen tree that has enormous ecological and socio-economic benefits. It is a tree species with multiple uses, including the ability to fix atmospheric nitrogen. Indeed, it contributes to increased soil nitrogen content, and consequently fosters the ecological restoration of steppes. It is one of the most promising forest trees in Mediterranean ecosystems, since all of its components are exploitable (leaves, flowers, fruits, wood, bark, and roots). The products of the carob tree have recently been treasured in terms of food industry (Boutasknit et al., 2020). Moreover, the pulp of pods and tegument are used to produce natural antioxidants and carob gum.

The carob tree is essentially present in semi-arid and arid areas, as well as coastal areas. Owing to its drought tolerance, as a response to water deficit, it develops morphological and physiological adaptation strategies by decreasing its leaf surface, making the leaves roll up (Batlle and Tous, 1997), and by developing the growth of its root system (Abdelkader et al., 2019). Thus, the ratio of dry matter between underground and aerial parts is increased (Essahibi et al., 2018). In view of its outstanding morphophysiological characteristics, such as salinity and drought

tolerance, in addition to its adaptation to poor soils (Batlle and Tous, 1997), carob trees are used for reforestation programmes in arid and degraded areas of Mediterranean ecosystems (Sakcali and Ozturk, 2004). Therefore, the selection and use of endemic species would improve the success rate of ecosystem restoration projects of artificial forest. The most appropriate area for investigating the photosynthetic response of plants to drought is the natural vegetation of Mediterranean ecosystems. Within this framework, the aims of the current study are: (1) to understand the water relations of *C. siliqua* under drought and to detect the acclimatisation mechanism of *C. siliqua* in the greenhouse after transplantation; (2) to assess the variations of photosynthetic response, stomatal control, and transpiration in three accessions under prolonged water stress; and (3) to identify the most tolerant and appropriate accessions for the recovery of degraded areas and for use in reforestation programmes.

2   Materials and methods

2.1   Plant material and experimental design

After determining and assessing the responses of seed germination of *C. siliqua* to temperature, water and salt stresses, and their combination (Zagoub et al., 2022), we focused on the response to drought stress generated by four different water treatments in these three accessions with the aim of simulating the average precipitation of Tunisian drylands, which are threatened by climate change. Seedlings of the three *C. siliqua* accessions were grown from seeds collected from three geographical locations in Tunisia. The origin of the three accessions studied, the climatic characteristics of the collection site, and their geographical locations are summarized in Table 1.

The relative humidity and monthly mean temperature varied from 56.6% to 70.0% and from 15.6°C to 29.6°C, respectively (Fig. 1). Information about the collection site was obtained from a meteorological station in Sfax. The three studied accessions were established through seedling during spring 2018 in plastic pots of 20 L, 30 cm in diameter, and 30 cm deep, under semi-controlled conditions and a greenhouse shelter. This greenhouse was located at the experimental station of the Institute of the Olive Tree of Sfax (34°43′N, 10°41′E) in central-eastern Tunisia. The soil used was sandy, composed of 85% sand, 10% silt, and 5% clay.

Table 1   Geographical origin and environmental conditions of the three accessions of *Ceratonia siliqua*

Accession	Code	Longitude	Latitude	Mean annual precipitation (mm)	Type of climate (Emberger, 1954)
Ichkeul	A1	09°40′14″E	37°08′24″N	600	Sub-humid
Melloulech	A2	10°59′96″E	35°11′93″N	300	Semi-arid
Matmata	A3	10°05′30″E	33°32′32″N	200	Hyper-arid
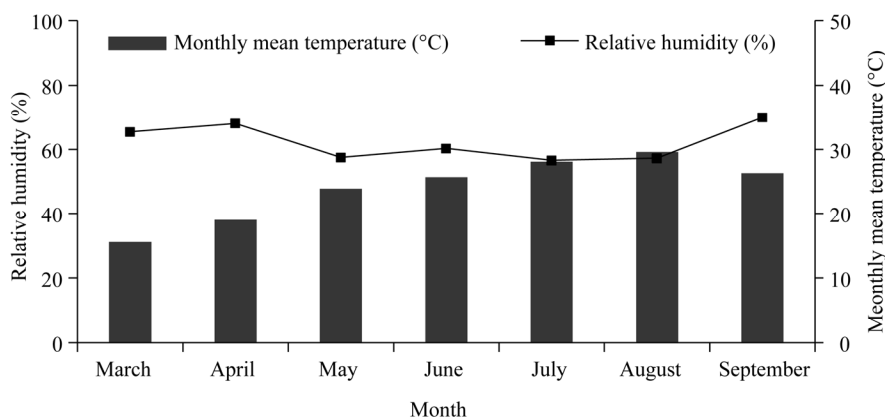
Fig. 1   Relative humidity and monthly mean temperature in the greenhouse during experimental period in 2020

All mature seeds of each accession sown in pots (six seeds of *C. siliqua* per pot) were treated with 98% concentrated sulphuric acid for 20 min at 24°C, then washed thoroughly with tap water and dried on a paper towel. During the first period of installation, individuals were regularly irrigated with tap water characterised by a pH value of 7.5, an electrical conductivity of 1.8 mS/cm, and containing 1.3 g of NaCl. The pots were moved arbitrarily each week to minimise position and edge effects. Two years after germination and seedling establishment, a single individual was maintained in each pot, and four water treatments were applied for seven months from March to September in 2020. The application of the treatments started one month before the beginning of the observation (i.e., in February). The four water treatments were Tc, T1, T2, and T3, which were 250, 180, 100, and 50 mm, respectively (Table 2). During this period, we measured the morphological and physiological parameters of seedlings under different water treatments. The experiment was conducted with 120 pots (40 pots for each accession and 10 replications per water treatment).

Table 2   Split irrigation amounts applied during experimental period

Code	Water treatment (mm)	Explanation of water treatments
Tc	250	This is average precipitation of ideal year under arid region of Tunisia, which has a probability of occurrence of 0.2% according to Floret et al. (1981).
T1	180	This is usual average precipitation in the arid region of Tunisia according to Le Houerou (1969).
T2	100	This is usual average precipitation of the upper Saharan bioclimate according to IPCC (2021).
T3	50	This is usual average precipitation of the lower Saharan bioclimate of Tunisia.

2.2   Morphological and physiological parameters

To investigate the response of *C. siliqua* to different levels of water stress, we measured morphological and physiological parameters every 2 weeks throughout the observation period (Table 3). Gas exchange parameters were determined from 09:00 to 11:30 (LST) using an IRGA (infrared gas analyzer) portable infrared CO_2/H_2O gas analyser (LCpro-32 070, ADC Bioscientific Ltd., Great Amwell, UK) on fully expanded leaves. Measurements were recorded on four leaflets for each individual. For each studied accession, a total of 5 individuals per treatment were selected to measure leaflet gas exchange. The remaining individuals (5 individuals per treatment) were used to measure predawn leaf water potential (Ψ_{pd}) and midday leaf water potential (Ψ_{md}), using a pressure chamber (Scholander et al., 1965). The time between excision and determination did not exceed 50 s. The soil water content (SWC) was estimated using a wet sensor (Delta-T Devices Ltd., Cambridge, UK), which is an indicator of drought intensity. Chlorophyll content was measured using chlorophyll metre (Minolta SPAD (single-photon avalanche detector)-502, Minolta Ltd., Osaka, Japan).

Table 3   Parameters measured during experimental period

Physiological parameter	Morphological parameter
Net CO_2 assimilation rate (A, μmol/(m²·s))	Plant height (H, cm)
Stomatal conductance (g_s, mol H_2O/m²·s))	Number of leaves formed (NL)
Transpiration rate (E, mmol H_2O/(m²·s))	Number of leaflets formed (Nl)
Internal CO_2 concentration (C_i, μmol/mol)	Number of branching formed (NB)
Predawn leaf water potential (Ψ_{pd}, MPa)	Chlorophyll content (SPAD value)
Midday leaf water potential (Ψ_{md}, MPa)	

Note: SPAD, single-photon avalanche detector.

2.4   Statistical analysis

To specify the separate and interactive effects of the three *C. siliqua* accessions on the response

variables related to physiological parameters, we performed variance analysis. The difference in physiological and morphological parameters was assessed by one-way analysis of variation (ANOVA). The comparison between the three studied accessions was carried out through the use of the Tukey's test. The statistical analysis of these data was undertaken using the excel software. Spearman's correlation analysis was conducted to establish the relationships between different measured morphological and physiological parameters as well as between the studied accessions and their functional traits. A two-way ANOVA was used to study the effects of the main factors (treatments, dates, and accessions) and their interactions on the plant response to stress. Differences between treatments for each accession and those between study months of each treatment per accession were determined by the Tukey's post hoc test at $P<0.05$ level.

3　Results

3.1　Effects of drought stress on morphological and physiological parameters of *C. siliqua*

3.1.1　Morphological parameters

For morphological traits, there were significant differences between different treatments for all measured parameters (Table 4). Concerning heights, at the end of experiment, the three accessions were 12.92, 21.96, and 14.25 cm under control treatment, respectively, for accessions A1, A2, and A3. Under water stress treatment, *C. siliqua* plants did not reach the heights recorded for their respective controls especially under T3 treatment. These values were 10.44, 11.52, and 11.82 cm for accessions A1, A2, and A3, respectively under T3 treatment (Fig. 2). *C. siliqua* decreased its growth process in response to water stress.

　　The number of leaves varied among the four treatments for all accessions. As shown in Figure 2, the highest number of leaves (9.42 (±1.28)) generated by accession A1 was higher than that of control plants, while the lowest number of leaves (5.16 (±0.51)) was recorded in the most stressed plants (T3). Accession A1, developed more leaves than the other two accessions. For accessions A2 and A3 of control plants, the values were 8.30 and 6.20 leaves, respectively, and 5.50 and 3.80 leaves for the most stressed plants, respectively. Similar to the number of leaves, the highest number of leaflets was found in control plants, while the lowest number was occurred in plants undergoing the most severe drought treatment. The values were 19.18, 25.12, and 12.98 leaflets for control plants, and 10.60, 12.80, and 11.12 leaflets for the most stressed plants in accessions A1, A2, and A3, respectively. Accessions A1 and A2 displayed a higher production of leaf biomass (number of leaves and leaflets) than accession A3. Analysis of variance revealed that the accession factor was very highly significant ($P<0.001$) for all studied morphological traits (Table 4). The water stress exerted a negative effect on plant growth and production. *C. siliqua* decreased its growth rate in response to increasing aridity.

3.1.2　Physiological parameters

As for the measured gas exchange parameters, there were significant differences among the three *C. siliqua* accessions (Table 5). The *A* dropped considerably under water stress for all accessions, as illustrated in Figure 3. The highest values were recorded for accessions A2 and A1 (3.40 and 3.36 μmol/(m²·s), respectively) for control plants. Under T3 treatment, the seedlings photosynthesis decreased by 56.55%, 52.95%, and 41.34% for accessions A1, A2 and A3, respectively, compared with control plants. Statistical analysis revealed a significant difference among different treatments ($P<0.05$). However, the decrease in accession A was associated with a significant difference ($P<0.05$), varying g_s in relation to the increase in water stress. Compared with control plants, these reductions were 54.17%, 54.55%, and 73.34% for accessions A3, A2, and A1, respectively, under T3 treatment. The stomatal opening was significant in accession A1 (0.45 mol H₂O/(m²·s)). In parallel to the decreases in *A* and g_s, significant reductions ($P<0.05$ for accessions A1 and A2) in transpiration rate (*E*) were recorded in *C. siliqua* accessions for different treatments. For T3 treatment, *E* was about 64.42% lower than that of control plants for accession A1. For accession A2, the decrease in *E* was approximately 44.24% lower than that of Tc, while this decrease reached up to 40.55% compared with control plants for accession A3.

Table 4 Two-factor analysis of variation (ANOVA) results for morphological traits of *Ceratonia siliqua*

Index	Source	*df*	*F*	*P*
Plant height	Accession	2	35.238	***
	Treatment	3	27.641	***
	Date	29	1.472	*
	Accession×Treatment	6	7.900	***
	Accession×Date	18	0.042	ns
	Treatment×Date	27	0.047	ns
	Accession×Treatment×Date	51	0.030	ns
Number of leaves	Accession	2	18.019	***
	Treatment	3	53.743	***
	Date	29	6.026	***
	Accession×Treatment	6	7.443	***
	Accession×Date	18	0.470	ns
	Treatment×Date	27	0.483	ns
	Accession×Treatment×Date	51	0.408	ns
Number of leaflets	Accession	2	32.593	***
	Treatment	3	30.410	***
	Date	29	5.952	***
	Accession×Treatment	6	5.850	***
	Accession×Date	18	0.766	ns
	Treatment×Date	27	0.460	ns
	Accession×Treatment×Date	51	0.191	ns
Number of branches	Accession	2	8.474	***
	Treatment	3	41.877	***
	Date	28	1.535	*
	Accession×Treatment	6	6.002	***
	Accession×Date	18	0.329	ns
	Treatment×Date	27	0.086	ns
	Accession×Treatment×Date	51	0.103	ns
Chlorophyll content	Accession	2	9.704	***
	Treatment	3	5.949	***
	Date	9	5.819	***
	Accession×Treatment	6	0.929	ns
	Accession×Date	14	2.516	***
	Treatment×Date	21	3.313	***
	Accession×Treatment×Date	42	1.475	*

Note: ns, not significant; *, $P<0.05$ level; **, $P<0.01$ level; ***, $P<0.001$ level.

Likewise, the internal carbon was affected by an increase in water stress for the three accessions studied. Indeed, the plants that received the most severe drought treatment displayed internal carbon rates that decreased by 49.46%, 22.89%, and 39.4%, respectively, for accessions A1, A2, and A3 compared with control plants. Thus, we deduced that drought has a negative effect on several metabolic activities, including *A*. The leaf water potential of *C. siliqua* decreased (becomes more negative) with gas exchange owing to the decrease in SWC (Fig. 3). The decrease in relative
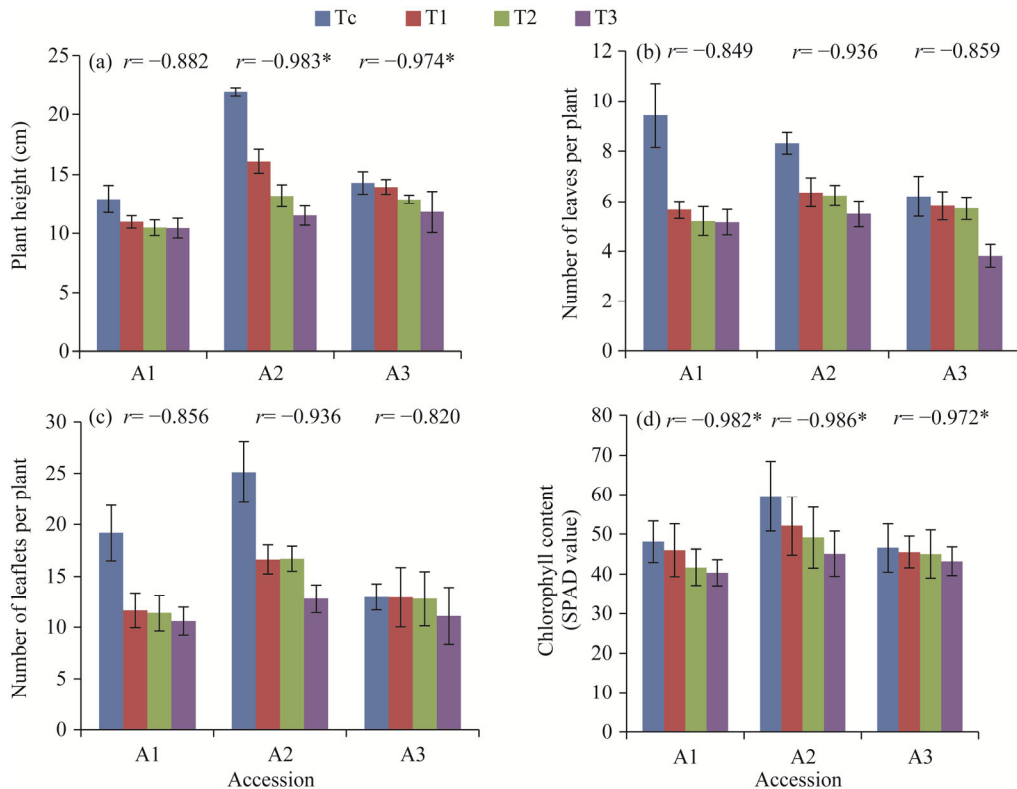
Fig. 2 Variation in morphological traits among *Ceratonia siliqua* accessions under different water treatments. (a), plant height; (b), number of leaves per plant; (c), number of leaflets per plant; (d), chlorophyll content. Tc, 250 mm/a; T1, 180 mm/a; T2, 100 mm/a; T3, 50 mm/a. SPAD, single-photon avalanche detector. *, $P<0.05$ level.

SWC brought about a reduction in leaf water potential for different treatments (T1, T2, and T3). In fact, a significant difference was observed between treatments in accession A2 (Fig. 5) and between accessions ($P<0.001$) (Table 6). For accession A2, Ψ_{pd} decreased significantly ($P<0.05$) from −0.96 MPa for control to −1.24 MPa for T3 treatment. In accession A3, Ψ_{pd} decreased from −1.06 to −1.50 MPa. However, the smallest reduction in Ψ_{pd} was recorded in accession A1, whose Ψ_{pd} value decreased from −1.01 to −1.24 MPa. Along with Ψ_{pd}, Ψ_{md} decreased progressively with the increase in water stress. With respect to this parameter, there was a difference between treatments for each accession. Indeed, for accession A1, the value of Ψ_{md} decreased significantly ($P<0.05$) from −1.97 to −2.83 MPa, and from −1.94 to −2.83 MPa for accession A2. For different treatments, the lowest value of Ψ_{md} was recorded in accession A3 under T3 treatment, reaching up to −2.56 MPa (Fig. 3).

3.2 Relationships between morphological and physiological parameters

The variations of Ψ_{pd}, Ψ_{md}, and SWC for the three *C. siliqua* accessions are respectively depicted in Figures 2 and 4. In case of water stress, all three *C. siliqua* accessions reduced their leaf water potential. The intensity of this decrease varied according to the origin of accessions and proved to be more important when more severe stress treatment was applied. Indeed, Ψ_{pd} and Ψ_{md} decreased as water stress increased, and varied from –0.96 to –1.50 MPa at sunrise and from –1.94 to –2.83 MPa at midday, respectively, under control and T3 treatment. For the three studied accessions, leaf water potential of control plants was always higher than that of T3 treatment. The difference being about 0.60 MPa for Ψ_{pd}, and around 0.90 MPa for Ψ_{md}. Leaf water potential followed changes in SWC for all studied treatments. Indeed, a positive correlation between Ψ_{pd} and SWC was observed for the three studied accessions (Fig. 4a).

Along with leaf water potential, gas exchange parameters such as stomatal conductance and

Table 5 Two-factor analysis of variation (ANOVA) results for gas exchange parameters of *Ceratonia siliqua*

Index	Source	df	F	P
A	Accession	2	15.243	***
	Treatment	3	17.538	***
	Date	7	9.350	***
	Accession×Treatment	6	2.895	**
	Accession×Date	14	4.972	***
	Treatment×Date	21	0.984	ns
	Accession×Treatment×Date	42	1.563	*
g_s	Accession	2	23.337	***
	Treatment	3	28.457	***
	Date	7	9.041	***
	Accession×Treatment	6	2.269	*
	Accession×Date	14	8.514	***
	Treatment×Date	21	3.309	***
	Accession×Treatment×Date	42	2.353	***
E	Accession	2	24.099	***
	Treatment	3	77.320	***
	Date	7	22.216	***
	Accession×Treatment	6	2.049	*
	Accession×Date	14	10.992	***
	Treatment×Date	21	5.416	***
	Accession×Treatment×Date	42	6.684	***
C_i	Accession	2	12.475	***
	Treatment	3	31.481	***
	Date	7	34.769	***
	Accession×Treatment	6	3.831	***
	Accession×Date	14	8.940	***
	Treatment×Date	21	1.964	**
	Accession×Treatment×Date	42	3.121	***

Note: A, net CO_2 assimilation rate; g_s, stomatal conductance; E, transpiration rate; C_i, internal CO_2 concentration; ns, not significant; *, $P<0.05$ level; **, $P<0.01$ level; ***, $P<0.001$ level.

transpiration rate were also sensitive to decreasing SWC (Figs. 3 and 4b). Significant differences in E and g_s were observed between treatments of each accession. For accession A1, the results revealed significant decreases in E and g_s, in response to decreasing SWC (Fig. 3). It can be detected that the physiological parameters are dependent on the variation of leaf water potential since the decrease of g_s follows the decrease of leaf water potential. This drop in leaf potential is closely related to the decrease in SWC. In addition, for the three *C. siliqua* accessions, the decrease of SWC induced a decrease of leaf water potential, generating a lower stomatal conductance. Concerning the chlorophyll content, highly significant differences were observed between accessions, and between treatments of each accession. Indeed, the results demonstrated that in accession A1, the chlorophyll content decreased from 48.13 (SPAD value) under Tc treatment to 40.40 under T3 treatment, from 59.60 to 45.12 and from 46.56 to 43.25, respectively, for both accessions A2 and A3. This decrease in chlorophyll content is strongly related to the increase in stress, as there is a positive and significant correlation (except for accession A3) between chlorophyll content and SWC. The strongest correlation between these two parameters was recorded in accession A2. The lowest significant correlation was observed in accession A3 (Fig. 4c).
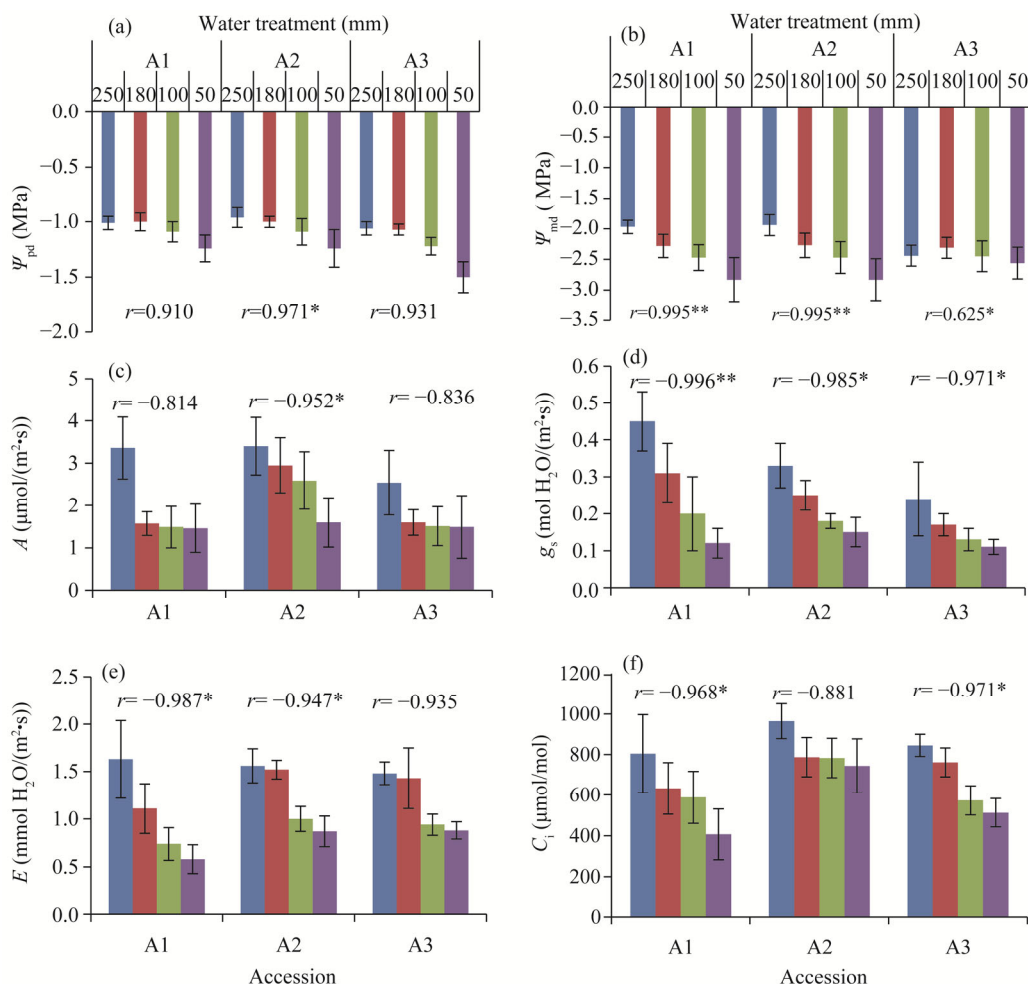
Fig. 3　Variation in gas exchange parameter and water potential among *Ceratonia siliqua* accessions under different water treatments. (a), Ψ_{pd}, predawn leaf water potential; (b), Ψ_{md}, midday leaf water potential; (c), A, net CO_2 assimilation rate; (d), g_s, stomatal conductance; (e), E, transpiration rate; (f), C_i, internal CO_2 concentration.

4　Discussion

4.1　Response of morphological trait of C. *siliqua* to drought

In arid and semi-arid areas, the most serious environmental stress on plant productivity, growth, and development is water stress induced by climate change (Passioura, 2006). Notably, the ability of plant to survive under stressful environmental conditions depends on its ability to receive stress, to create and transmit the signal to different parts of plant, and thus to trigger a set of physiological and morphological changes (Ahmadizadeh et al., 2011). Our results illustrated the existence of significant differences between different treatments for all studied morphological parameters (Table 4). Accessions A1 and A2 displayed higher leaf biomass production than accession A3, and following the application of water stress, the growth of *C. siliqua* was influenced by the increase in stress. These morphological responses to water stress reflect the adaptation of this species to environmental conditions. Therefore, considering the variation in temperature and air humidity (Fig. 1), growth and leaf development are influenced not only by this variation, but also by the increase in water stress. The plants reached the maximum leaf growth before the beginning of water stress, similar to the results reported by Nunes et al. (1992), since the leaves of *C. silique* were formed in spring, and they became more mature before drought severity increased. In Mediterranean ecosystems, although water availability is a determining

Table 6　Two-factor analysis of variation (ANOVA) results for leaf water potential and SWC of *Ceratonia siliqua*

Index	Source	df	F	P
Ψ_{pd}	Accession	2	19.792	***
	Treatment	3	113.513	***
	Date	7	88.768	***
	Accession×Treatment	6	4.439	***
	Accession×Date	14	3.391	***
	Treatment×Date	21	9.809	***
	Accession×Treatment×Date	42	1.961	***
Ψ_{md}	Accession	2	3.582	*
	Treatment	3	159.107	***
	Date	7	293.495	***
	Accession×Treatment	6	11.729	***
	Accession×Date	14	8.902	***
	Treatment×Date	21	13.709	***
	Accession×Treatment×Date	42	4.778	***
SWC	Accession	2	9.825	***
	Treatment	3	102.434	***
	Date	7	31.546	***
	Accession×Treatment	6	1.121	ns
	Accession×date	14	0.803	ns
	Treatment×Date	21	4.994	***
	Accession×Treatment×Date	42	1.491	*

Note: Ψ_{pd}, predawn leaf water potential; Ψ_{md}, midday leaf water potential; SWC, soil water content; ns, not significant; *, $P<0.05$ level; **, $P<0.01$ level; ***, $P<0.001$ level.
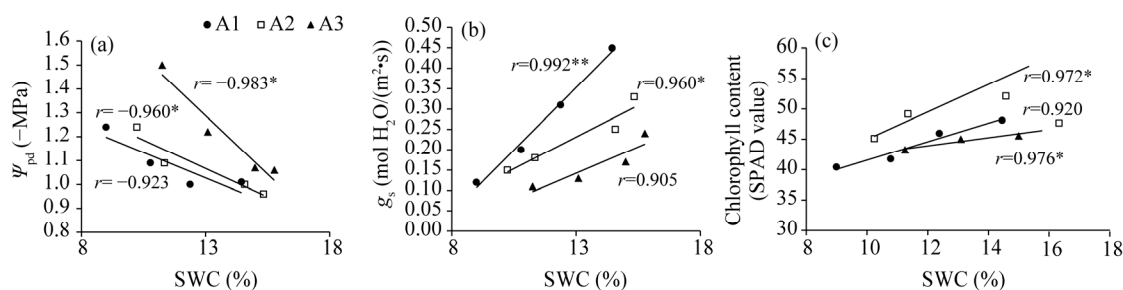
Fig. 4　Relationships among physiological, morphological, and SWC (soil water content) parameters of *Ceratonia siliqua* accessions (A1–A3). (a), relationship between predawn leaf water potential (Ψ_{pd}) and SWC; (b), relationship between stomatal conductance (g_s) and SWC; (c), relationship between chlorophyll content and SWC. SPAD, single-photon avalanche detector. *, $P<0.05$ level; **, $P<0.01$ level.

factor in tree growth (Correia et al., 2001), environmental factors also play a key role in plant growth (Passioura, 2006). Similar results were found for two *C. siliqua* cultivars (Espargal and Mulata) subjected to water stress (Correia et al., 2001).

In addition, the reduced rate of photosynthesis during drought limits CO_2 uptake, thus leading to reduced leaf growth and development, as growth and carbon assimilation are severely limited by drought stress (Zhang et al., 2019). As drought becomes increasingly severe, trees become more vulnerable, which may cause ecophysiological weakening of forest ecosystems (Roibu et al., 2020). Therefore, two highly important abiotic factors, namely temperature and water availability,

need to stay within certain ranges for maximum plant growth. The comparison of data concerning the height of plants of the three accessions showed significant differences between multiple accessions and among water treatments (Table 5; Fig. 2). Accession A2 displayed the highest height, followed by accessions A3 and A1. This result was similar to the findings reported by Touckia et al. (2015), who emphasised that growth varies according to accession. The application of water stress treatment entailed greater reductions in height in accession A2, while the accession least affected by the severity of water stress was A1. In this respect, tolerance to this stress leads to changes at the cellular level, as it causes a decrease in cell enlargement and growth (Shao et al., 2008). Notably, water stress has a great influence on the growth and production of plants at several levels, from the cell to the community (Colom and Vazzana, 2001). Correia et al. (2001) unveiled that the quality and quantity of plant growth is dependent on cell division, differentiation, and enlargement. All of these mechanisms are affected by water stress. Facing the tremendous increase in stress severity, the seedlings had considerably reduced their leaf surface area from 3.41 to 5.12 cm^2 with the aim of decreasing transpiration by limiting their leaf growth and increasing their root system to better exploit soil water. Indeed, cell expansion can only occur if the turgor pressure is higher than the cell wall yield threshold. Therefore, stress inhibits growth and cell expansion due to low turgor pressure (Karthikeyan et al., 2007). Kusaka et al. (2005) highlighted that drought has a strong impact on most of the growth parameters of aboveground and belowground parts of agricultural and forest trees. This further confirms our findings since drought reduced the growth of the three *C. siliqua* accessions. These morphological aspects thus reflect and reinforce the plant's ability to cope with environmental conditions.

Facing hydric constraint, the leaves and leaflets of the three *C. siliqua* accessions were not affected in the same intensity. With the increase in stress severity, accessions A1 and A2 had a reduced number of leaves and leaflets compared with accession A3, allowing them to withstand the increased drought. However, the number of leaves produced under T3 treatment was still lower than that of control plants. Similarly, Lo Gullo et al. (1986) found that the reduction in leaf growth in spring allowed young carob leaves to tolerate the impacts of drought since they were vulnerable to water stress. In fact, they undergo significant reductions in symplasmic water content (Correia et al., 2001). Nunes et al. (1992) asserted that *C. siliqua* leaves were formed in the spring and thus reached maturity when the cell wall became less elastic and the severity became increasingly intense. As far as our research is concerned, the reduction in the production of new leaves by accessions demonstrates a drought avoidance mechanism that does not display the same intensity between different accessions. A decrease in growth is the consequence of water stress on morphological behaviour of plant, which can be advantageous. Indeed, with a decrease in growth, the leaf surface is reduced, and consequently, transpiration also drops due to rolling of plant leaves (Grandi et al., 2021). Under stress conditions, the chlorophyll content decreases, and this fact has been confirmed by several authors. In this line, Viera et al. (1991) emphasised that the decrease in chlorophyll content could trigger a decrease in plant growth parameters under water stress conditions. This finding is in accordance with our study's outcome since highly stressed plants (T3) had a lower chlorophyll content, which thus coincides with a lower growth rate (Fig. 2). This reduction may be attributed to a decrease in chlorophyll biosynthesis or degradation (Abdelkader et al., 2019).

4.2   Response of physiological trait of *C. siliqua* to drought

Aiming to cope with water stress, our plants developed several defence mechanisms. One of the operational mechanisms that plays an important role in terms of water balance modelling is stomata, which controls gas exchange and contributes to the adaptation of plant to stress (Devireddy et al., 2020). Within this framework, following the application of water stress, our seedlings significantly reduced their A compared with control plants. However, this decrease in A was associated with reductions in E and g_s ($P<0.05$), inducing a subsequent reduction in carbon fixation rate relating to the increase in water stress (Fig. 3). The main mechanism by which plants control their water loss is stomatal closure (Caser et al., 2018). Thus, to decrease hydraulic failure,

plants close their stomata, but this reduces the intracellular concentration of CO_2 and decreases water loss. In fact, several tree species reduce their stomatal conductance once exposed to drought conditions by reducing water consumption per assimilated carbon, thus improving their water use efficiency (Rouina et al., 2007; Dayer et al., 2020).

Yousfi et al. (2016) found that plant physiological responses were strongly influenced by the duration and intensity of drought. This is consistent with our study, as most significant reduction in gas exchange parameters was observed in most severe water stress treatment (Fig. 3). Boutasknit et al. (2020) found that stomatal conductance was significantly reduced in stressed *C. siliqua* plants. Therefore, to decrease transpiration, *C. siliqua* reduced their stomatal conductance. This phenomenon corresponds to a crucial strategy for maintaining sufficient levels of hydration at tissue level and turgor at cellular level (Essahibi et al., 2018). The decrease in stomatal conductance reduces water consumption through transpiration, which has an intrinsic impact on preventing the drastic effect of drought on photosynthesis and plant growth (Ramalho et al., 2000). The net assimilation rate reached the highest values, i.e., 3.40 and 3.36 $\mu mol/(m^2 \cdot s)$ in accessions A2 and A1, respectively, under control treatment. This high rate of photosynthesis coincides well with the increase in carbohydrate consumption, referring to the high growth rate of plants (Ouzounidou et al., 2012). Subsequently, with the increase in water stress (T3 treatment) and average greenhouse temperature, and the decrease in carboxylation efficiency, the photosynthetic rate decreased considerably in the three accessions. This decrease was affected in part by stomatal closure (Kchaou et al., 2013) and in other parts by concomitant damage related to photoinhibition generated during dry season (Ouzounidou et al., 2012).

The internal CO_2 concentration in stressed accession was significantly lower than that of control plants. It decreased by 49.46%, 22.89%, and 39.4% for accessions A1, A2, and A3, respectively, compared with control plants. Poulos et al. (2007) elucidated that during drought, a decrease in photosynthesis reduces CO_2 uptake, which subsequently limits leaf growth. According to Yamaguchi-Shinozaki and Shinozaki (2006), the closure of stomata accompanied by a decrease in conductance following drought stress represents a drought tolerance factor for plants. Following the increase in water stress, the relative water content of soil and stomatal conductance decrease considerably. This results in the closure of stomata to minimise water loss (Pita et al., 2005).

The three accessions displayed two different behaviours when facing with water stress. Kozlowki and Pallardy (2002) clarified that the response to stress differs among species, genotypes, and even among parts of the same plant. There are intra-specific differences in stomatal sensitivity, which is indicative of different adaptations to applied water stress related to genetic variation of the three accessions. Under drought conditions, photosynthesis, osmotic adjustment, and stomatal conductance become lower (Ladjal et al., 2007). During water stress, plants that adopt a water expenditure strategy to avoid water stress are unable to minimise water losses and exhibit high levels of hydration when exposed to external water stress. This can be accounted for rapid extraction of water from soil to recover water losses resulted from transpiration, which leads to a strong reduction in water potential of accessions A1 and A2 (Ozturk et al., 2010). According to Angelopoulos et al. (1996), *C. siliqua*, a Mediterranean plant, adopts this strategy to cope with water stress. The relationship between transpiration and photosynthesis requires a certain level of stomatal opening. This is essential for the success of species in dry environments (Ozturk et al., 2010). Grzesiak et al. (2006) considered that leaf water potential is a direct indicator of water status and the degree of drought in different plants. Our results revealed that, under drought stress, leaf water potential decreased in all three accessions, and accession A3 developed the lowest potential compared with the other two accessions (Fig. 3). This decrease was mainly ascribed to the reduction in relative SWC. Oren et al. (1999) argued that multiple plants reduced their stomatal conductance to cope with a decrease in water potential. In this context and with the most severe water stress treatment (T3), the capacity of the tissues of the plants to lose water by transpiration brought about a decrease in leaf water potential, which generated an increase in the concentration of cellular solutes (Correia et al., 2001). During severe

drought, Ψ_{pd} values of *C. siliqua* ranged from −0.40 MPa (Lo Gullo and Salleo, 1988) to −1.10 MPa (Correia and Martin-Loucao, 1995). As for our accessions, Ψ_{pd} decreased as water stress increased, ranging from −0.96 to −1.50 MPa for the three studied accessions. Thus, this species can maintain a high predawn potential during dry periods in view of deep rooting. During periods of high evaporative demand, the maintenance of high leaf potential can be affected by limiting transpiration, which is achieved through an efficient xylem transport system (Correai et al., 2001). The minimum water potential decreased to −2.90 MPa at the end of September, which is confimed by Correia and Martins-Loucao (1995). This species is characterised by mesomorphic leaves that display variations in daily water potential resulting from rapid changes in turgor pressure. This variation is due to a change in relative air humidity, temperature, and soil water availability (Ozturk et al., 1983). Lo Gullo et al. (1986) suggested that sclerophyllous species, such as carobs, could extract water from soil rapidly to recover water losses. From this perspective, stress tolerance can be achieved through a rapid change in the plant water potential. These changes are produced by a decrease in turgor when facing water stress.

In the Mediterranean ecosystems, *C. siliqua* is considered a model of drought tolerance (Ozturk et al., 2010). This species adopts several strategies to cope with water shortages, such as water-spreading strategy (Correia et al., 2001). Correia et al. (2001) pointed out that the strategy most adapted by *C. siliqua* to cope with drought is avoidance. Indeed, the avoidance of dehydration results from a balance between capacity of water absorption by roots and its distribution in plant tissues and stomatal movements (Augé et al., 2016). These accessions are considered drought-escaping, and it can be concluded that this species can avoid water stress through its genotypic variability in developing drought-avoidance mechanisms. In this respect, Gill and Tuteja (2010) asserted that during water stress, a decrease in relative SWC led to a water deficit in plant, which could lead to its death. Furthermore, at cell level, stress is translated by an increase in concentration of solute, causing a change in cell shapes with a loss of turgidity (Shao et al., 2008). Consequently, it causes alteration at membrane level and protein denaturation (Shao et al., 2008). Therefore, this species has great plasticity since it uses a different adaptation mechanism to prevent water loss.

Climatic variation is likely to produce an effect on productivity and photosynthesis. However, this effect varies according to not only the areas in relation to existing climatic conditions but also to adaptive potential of species (Niinemets et al., 2009). This species tolerates different levels of water stress. Therefore, it is suitable for natural reforestation of arid environments in the Mediterranean ecosystems. It can also maintain water balance under severe environmental conditions (Ouzounidou et al., 2012). The carob tree exhibits high productive potential during periods of drought. Therefore, it is recommended for agricultural production and planting (Ouzounidou et al., 2012).

5   Conclusions

With increasing climate change, knowledge of plant growth is essential under water deficit conditions to assess rangeland productivity. In this context, *C. siliqua* accessions showed a high ability to tolerate drought. Under this severe water stress, the greatest reduction in morphological parameters was recorded in accessions A1 and A2 compared with accession A3. Accessions A1 and A2 were more tolerant than accession A3. They also exhibited the lowest leaf water potential, photosynthesis, and stomatal conductance. The decrease in photosynthesis and gas exchange activity of *C. siliqua* was also associated with inhibition of Rubisco activity, lower water use efficiency with increasing temperature, decreasing CO_2 levels, and rapid leaf dehydration at negative water potential. This species uses the avoidance mechanism to minimise water loss through stomatal closure and to optimise root uptake. These results indicate that this species adopts an avoidance strategy to cope with drought. This strategy ensures good regulation of plant gas exchange under arid conditions. A close relationship of SWC with plant growth and physiological processes was recorded for all three accessions of *C. siliqua*. *C. siliqua* showed greater plasticity with respect to different climatic conditions. Thus, the species is likely to be

robust to future climate changes. It can be recommended for the restoration of degraded rangelands in arid environments.

Acknowledgements

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